Wetlands
https://doi.org/10.1007/s13157-020-01338-w

GENERAL WETLAND SCIENCE

# Salinity, Water Level, and Forest Structure Contribute to Baldcypress *(Taxodium distichum)* Rhizosphere and Endosphere Community Structure

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Received: 23 March 2020 / Accepted: 3 July 2020

## Abstract

As rising sea levels alter coastal ecosystems, there is a pressing need to examine the effects of saltwater intrusion on coastal communities. Using 16S Illumina profiling, we characterized the communities of baldcypress tree (*Taxodium distichum*) root endosphere and rhizosphere soil bacteria. Our study utilized established sites along salinity and flooding gradients in the United States of Georgia, Louisiana, and South Carolina. We hypothesized that environmental variables, namely salinity and water level, as well as distance between sites would be correlated with baldcypress-associated rhizosphere and root endosphere bacterial communities. We found that geographic distance correlated with rhizosphere but not root endosphere bacterial communities, suggesting that the trees may have stabilized their endosphere communities via recruitment of a more specific suite of taxa from the surrounding soil. Mean water level, mean salinity, and the volume of woody debris were associated with both endosphere and rhizosphere bacterial communities in baldcypress trees. The density of host trees was also associated with endosphere community composition. Our study is the first to use 16S Illumina sequencing to characterize bacterial communities in baldcypress trees— a key restoration species in coastal swamp ecosystems under threat from rising sea levels.

**Keywords** Coastal forested wetlands · Endosphere · Endophytic bacteria · Rhizosphere · Salinity · Soil bacteria

## Introduction

Sea level rise, storm surge, and subsidence all contribute to saltwater intrusion into the freshwater forested wetlands (swamps) of the Gulf and Southeastern coastal regions of the United States. As saltwater moves inland, swamps are being converted to marsh, where only standing dead or stressed trees remain among an understory of saltwater-adapted plant species (Krauss et al. 2009; Shaffer et al. 2009). Many swamp ecosystems are dominated by the deciduous conifer, *Taxodium distichum* (hereafter baldcypress) which is able to persist in a range of conditions associated with a healthy, dynamic, coastal landscape. For instance, baldcypress is moderately salt tolerant (up to 2 ppt (parts per thousand) salinity) and can also live in dry conditions or permanently, periodically, seasonally, or tidally flooded locations (Allen et al. 1996). The wide geographic range of baldcypress, as well as its ability to live across a spectrum of environmental conditions make it an ideal candidate species for examining plant-associated microbial community composition along gradients that are shifting due to saltwater inundation brought about by sea level rise. In addition, baldcypress is currently used in large-scale restoration efforts of degraded wetlands along the Gulf Coast for coastal protection (CPRA Coastal Master Plan 2017), further motivating this study as changes in the associated microbial communities can potentially impact its persistence or ability to adapt to changing environments.

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**Electronic supplementary material** The online version of this article (https://doi.org/10.1007/s13157-020-01338-w) contains supplementary material, which is available to authorized users.

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Two prominent groups of plant-associated bacteria are endophytes (bacteria that live entirely within the tissues of their host plants in the endosphere) and rhizosphere bacteria (which reside in the layer of soil directly surrounding the roots) (Wilson 1995; Friesen et al. 2011; Philippot et al. 2013). Both root endosphere and rhizosphere bacteria contribute to nutrient uptake, and increased growth, as well as a plant's abilities to respond to abiotic stressors such as temperature changes, flooding, and salinity (Rosenblueth and Martínez-Romero 2006; Philippot et al. 2013; Ryan et al. 2016; Yuan et al. 2016). Despite these known roles in plants' phenotypes and, in turn, ecological interactions and community dynamics (Wilson 1995; Friesen et al. 2011; Christian et al. 2015), what drives community composition of these bacterial groups remains underexplored, especially for plants in wetland and coastal ecosystems. Recent studies on coastal ecosystems suggest that bacterial communities are largely influenced by environmental factors such as salinity (Morrissey and Franklin 2015; Li et al. 2019), with higher salinity suppressing microbial function and changing the sediment microbial assemblages (e.g., Jackson and Vallaire 2009). Geographic distance, however, can influence the assembly of plant-associated microbes regardless of the environment as some bacterial taxa might exhibit dispersal limitation (e.g., Barreto et al. 2014).

Krauss et al. (2009) examined site condition, structure, and growth of baldcypress trees along salinity gradients in the southeastern United States (Fig. 1). Long-term, background data from these established sites offer a unique opportunity to examine the influence of a suite of environmental variables as well as geographic distance on plant-associated microbes. Our research builds upon Krauss et al. (2009) by characterizing the bacterial communities in the endosphere and rhizosphere of these same baldcypress trees.

In this study, we examine baldcypress root endosphere and rhizosphere bacterial community patterns with respect to environment and spatial distance across the southeastern US. We hypothesized that both geographic distance between host trees as well as environmental variables associated with saltwater inundation would influence baldcypress-associated rhizosphere and root endosphere communities and predicted that the endosphere community would be less affected by geographic distance than the rhizosphere community because of the additional biotic filter imposed by the host tree. We characterized baldcypress root and rhizosphere bacterial communities and asked the following questions: 1) Is bacterial community composition correlated with environmental variables and/or geographic distance? 2) Which environmental factors explain a significant amount of the variation in bacterial community composition? Understanding how bacterial communities are structured in association with this key restoration species may help us to more accurately predict how microbial communities will shift due to saltwater inundation brought about by sea level rise.

# Methods

## Site Descriptions

Baldcypress trees were sampled at sites along landscape gradients in the southeastern US (Fig. 1). These sites, which range from freshwater to oligohaline (salinity ranged from 0.1 to ~3.4 ppt), were established by the USGS (United States Geological Survey) and Clemson University in 2004 and have been continuously monitored for environmental data such as water level, salinity, and tree growth to provide long-term data on how *T. distichum* swamps are affected by climate change-induced salinization and flood regimes (Krauss et al. 2009). South Carolina (SC) and Georgia (GA) sites were strongly tidal and were located along transects associated with rivers and tidal creeks (Waccamaw River and Savannah River, respectively). Each transect had one degraded site established alongside a marsh (salt site), and two sites located progressively upstream to represent moderately degraded (mid site) and healthy (fresh site) stands for a total of three sites per transect (Fig. 1). Each site was comprised of two 20 × 25 m plots. Louisiana's two sites (LA) were associated with more "complex systems of interconnected bayous and open water bodies" (Krauss et al. 2009) and had periodic wind tides. There are six Louisiana sites, three fresh and three salt, all hydrologically independent of each other.

## Environmental Measurements

Within each plot, temperature, salinity and conductivity were measured manually from four salinity wells every four months in Louisiana and monthly in South Carolina and Georgia. Water level (the height of the water from the ground surface) was measured hourly by logging water level recorders (Vented pressure water level data logger, INFINITIES USA, INC., Port Orange, Florida). To characterize environmental conditions for each sample, we averaged measurements over the six to nine months preceding our collections. Baldcypress tree density (ha-1) was calculated based on the number of trees (individual >10 cm diameter at breast height [dbh]). Woody debris volume ($m^3$/ha) was measured as the volume of downed wood in contact with the soil and up to 1 m above the soil surface (Krauss et al. 2018).

## Sampling

In June of 2015, we collected root and rhizosphere samples from mature baldcypress trees along the South Carolina and Georgia transects. The Louisiana sites were sampled in March of 2016. We collected samples from five trees per site with the exception of one site, where we were only able to access three trees ($n = 58$ total trees). From each tree we collected a half liter Ziploc bag full of accessible roots from three points around the

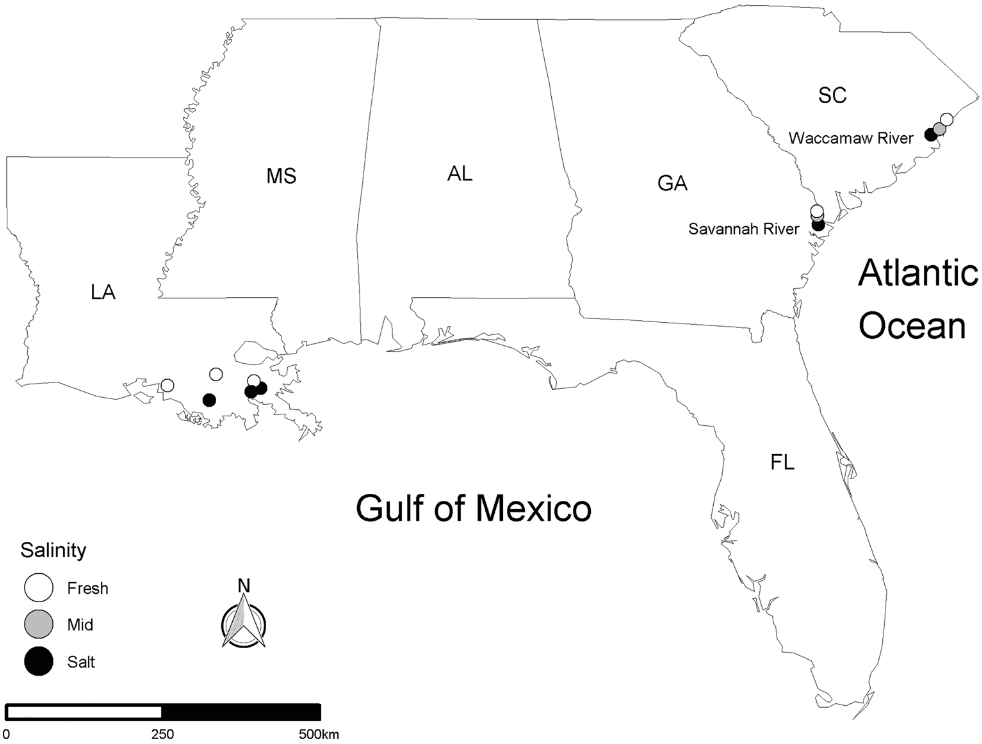


**Fig. 1** Map showing the location of the twelve sites established by Krauss et al. (2009). Each site was comprised of two 20 × 25 m plots and is classified according to salinity level (fresh, mid, and salt)

tree. Rhizosphere samples were obtained by using a gloved hand to pull soil from directly around a belowground or submerged root clump and placed into a sterile 50 mL falcon tube.

## Processing

We sterilized the roots using the protocol described in Mukasa Mugerwa et al. (2013) by submerging roots in 70% EtOH for 10 s, 50% Clorox (3.125% sodium hypochlorite) for 2 min and then rinsing twice with sterile deionized water. We used sterile scissors to cut the roots into small ~2 mm long pieces. These pieces were transferred into empty microtubes and placed in -20 °C freezer for downstream processing.

## Sequencing

We used MoBio PowerPlantPro DNA and MoBio PowerSoil DNA isolation kits (Qiagen, Hilden, Germany) to isolate DNA from the root endopshere and rhizosphere soil, respectively. Bacterial 16S ribosomal DNA (region V5-V6) was amplified with 799F and 1115R primers and tagged with an 8 bp barcode indices as described in Hanshew et al. (2013) and Kembel et al. (2014). The PCR products were normalized with SequelPrep plates, pooled, and concentrated with Agencourt AMPure beads. Amplicons were sent to Genewiz Inc. and sequenced on an Illumina MiSeq instrument using 300 bp paired-end sequencing.

## Bioinformatics

QIIME (Caporaso et al. 2010) was used to demultiplex and quality-filter the sequences. Sequences were dereplicated and clustered into Operational Taxonomic Units (OTUs) at a 97% threshold, in an open reference manner using UCLUST and assigned taxonomy with the GreenGenes 13_5 reference sequences (McDonald et al. 2012). Chimera detection and removal was conducted with the uparse algorithm in USEARCH (Edgar 2013). Mitochondrial 16S sequences were filtered out as well as samples with low sequencing success (OTU count <500). Singleton OTUs (OTUs with sequence count = 1) were excluded from analyses. Prior to all analyses, we used rrarefy function in the R package vegan (Oksanen et al. 2013) to rarefy our data to $n = 1500$. Raw sequences were submitted in the NCBI Genbank Sequence Read Archive (PRJNA638050).

## Statistical Methods

Collinearity of environmental variables was determined in R v 3.6 (R Core Team 2016) by first clustering variables using the Ward's d minimum variance. From this, temperature and conductivity were removed as they were correlated with water level and salinity, respectively. Thus, the variables used in our analyses were: tree density, woody debris volume, salinity, and water level. Environmental data were natural log-transformed, and all continuous variables were scaled to standard z-scores, with mean of 0 and standard deviation of 1 in order to compare estimates among variables. All analyses were conducted at the OTU level and separately for endosphere and rhizosphere OTUs.

### Geographic Distance and Bacterial Community Structure

We examined the relationship between geographic/spatial distance and bacterial community composition relative to environmental variables by conducting a Multiple Regression on distance Matrices (MRM) analysis of pairwise community similarities (Bray Curtis) as a function of geographic distance (Lichstein 2007). We aggregated OTUs from 3 to 5 trees at the site level, summed the OTU abundances, and measured pairwise site dissimilarity as an abundance-weighted, Bray-Curtis. We used the distGeo function in the geosphere package (Hijmans 2015) in R v.3.4 (R Development Core Team 2016) to calculate the pairwise geographic distances between plots. We also included the Euclidean differences in the environmental parameters to control for effects of environmental differences. Similarity matrices were regressed against the geographic distance matrix and the Euclidean distance matrices using ecodist (Goslee and Urban 2007).

### Environmental Variables and Community Composition

We conducted a distance-based redundancy analysis (Db-RDA) in the R package vegan (Oksanen et al. 2013) on the Bray-Curtis dissimilarity matrix of both rhizosphere and root endosphere community data at the OTU level. We used the ordistep function to perform forward model selection, determining if any of the following variables explained the bacterial communities: temperature, woody debris volume, tree density, salinity, and water level. Models were then reduced by removing non-significant terms sequentially. We conducted the analyses first across all samples, then at the transect level (within each transect, e.g., Waccamaw) in order to account for the sampling locations/space.

### Environmental Variables and Community Composition across Taxonomic Levels

We examined shifts in bacterial community compositions at higher taxonomic levels (e.g., class, order, etc.) across transects in order to assess whether shifts at OTU level are mirrored by changes at higher taxonomic levels. For a good resolution of emergent patterns, we focused the analyses on only three taxonomic levels: class, order and family. We collapsed OTUs at each taxon level, then summed the sequence abundances of each OTU to that level, with unidentified OTUs lumped together. In order to assess shifts in bacterial community compositions at each taxon level, we conducted perMANOVA analysis at transect level using the Bray-Curtis distance matrix calculated for each taxon level, with transect, temperature, woody debris volume, tree density, salinity, and water level as well as the interaction of salinity and water level as predictor variables. We used the adonis function in vegan. We further visualized changes in abundant taxa across salinity levels within each transect and site by plotting the abundances of taxa at the family level in ggplot2 (Wickham 2016).

### Diversity/Richness/Abundance

We calculated Shannon diversity in the R v.3.4 (R Development Core Team 2016) package vegan (Oksanen et al. 2013) using the rare.d function and OTU richness (i.e. number of OTUs present within a community) using the rich.vegan function. We ran Mann Whitney U tests in PAST (PAlaeontological STatistics) (Hammer et al. 2001) to compare diversity among sites, transects and combinations of endosphere and rhizosphere communities in sites and transects. We ran a Kruskal-Wallace test to compare diversity between endosphere vs. rhizosphere communities. We used the multivariate statistical framework MaAsLin (Multivariate Association with Linear Models) (Morgan et al. 2012) to find associations between relative abundance of specific microbial taxa and environmental data. All MaAsLin analyses were performed at the Class level separately for rhizosphere and endosphere, which allowed for best resolution of emergent patterns.

## Results

### Geographic Distance and Bacterial Community Structure

Rhizosphere bacterial communities became more dissimilar with increasing geographic distance ($r = 0.06$, $p = 0.02$) (Fig. 2). The root endosphere bacterial communities showed a trend towards dissimilarity with increasing geographic distance but was not significant ($r = 0.005$, $p = 0.58$). Bacterial community dissimilarity matrices were regressed against the geographic distance matrix and the Euclidean distance matrices for the environmental variables to determine that the observed effect of distance was not correlated with differences among plots with respect to environmental variables.

### Environmental Variables and Community Composition

In rhizosphere bacterial communities, mean water level, mean salinity, and woody debris volume were selected as significant explanatory variables at the site level, which explained a significant amount of variation in the model (77.4%) using the

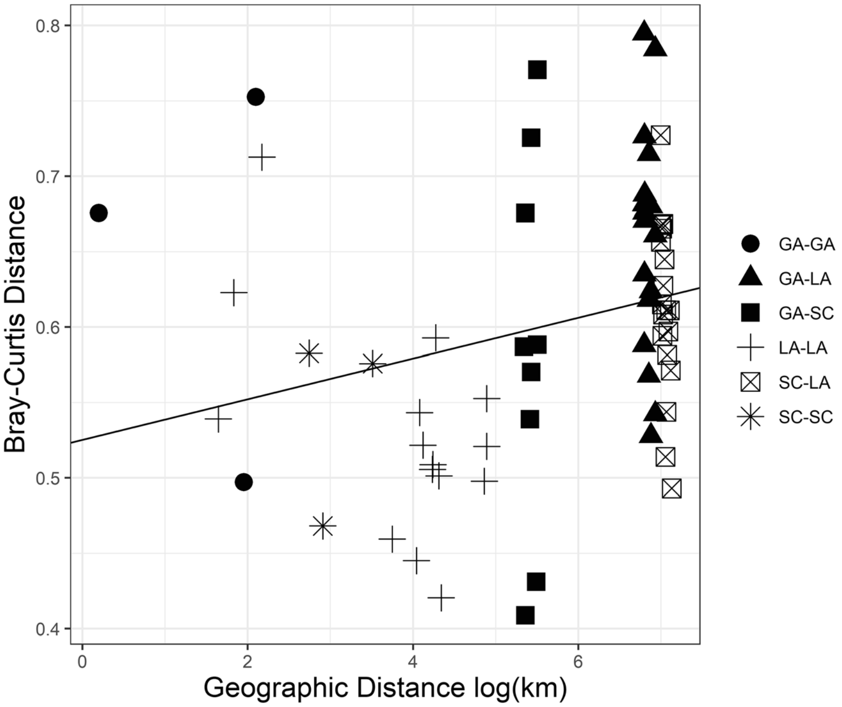


**Fig. 2** Rhizosphere bacterial communities were more dissimilar with increasing geographic distance (r = 0.06, $p = 0.02$). Points represent pairwise comparisons between sites and are described at the state level (Louisiana, Georgia, South Carolina). The further apart the two points are the greater the unifrac distance between the bacterial communities. For example, the longest geographic distance will always be LA-SC. Those points show that the unifrac distance (between centroids of their clouds of microbial communities) is variable and is greater than SC-SC comparisons

Bray-Curtis dissimilarity matrix in the DB-RDA analysis at OTU level (Table 1, Fig. 3). This was accompanied by shifts in bacterial taxa across taxonomic levels (i.e. from class to family level, Table S1). At the transect level, the magnitude of the environmental variables varied widely (Fig. S1). For instance, all four environmental variables were selected as significant explanatory variables in Barataria by the model, while none of the variables significantly explained bacterial community composition in Terrebonne (both transects were located in Louisiana). Woody cover and water level were selected as explanatory variables for Waccamaw transect in South Carolina while woody cover and water level were selected for the Georgia site (Savannah transect) (Fig. S1).

For root endosphere communities, mean water level, mean salinity, woody debris, and tree density were variables which explained a significant amount of variation (75.4%) in the model using the Bray-Curtis dissimilarity matrix (Table 1, Fig. 4). Similar to rhizosphere, at the transect level the environmental variables that had the strongest influence in explaining variations among endosphere communities differed. Water level and salinity showed a significant amount of variation in Savannah (Georgia) while water level and woody cover were identified as significant variables in Barataria in Louisiana (Fig. S2).

**Table 1** Distance-Based Redundancy Analysis (Db-RDA) results and comparison between environmental variables correlated with bacterial community composition of endosphere ($F_{model} = 2.94$, df = 4, $p = 001$) and rhizosphere ($F_{model} = 3.35$, df = 4, $p = 001$) across all samples

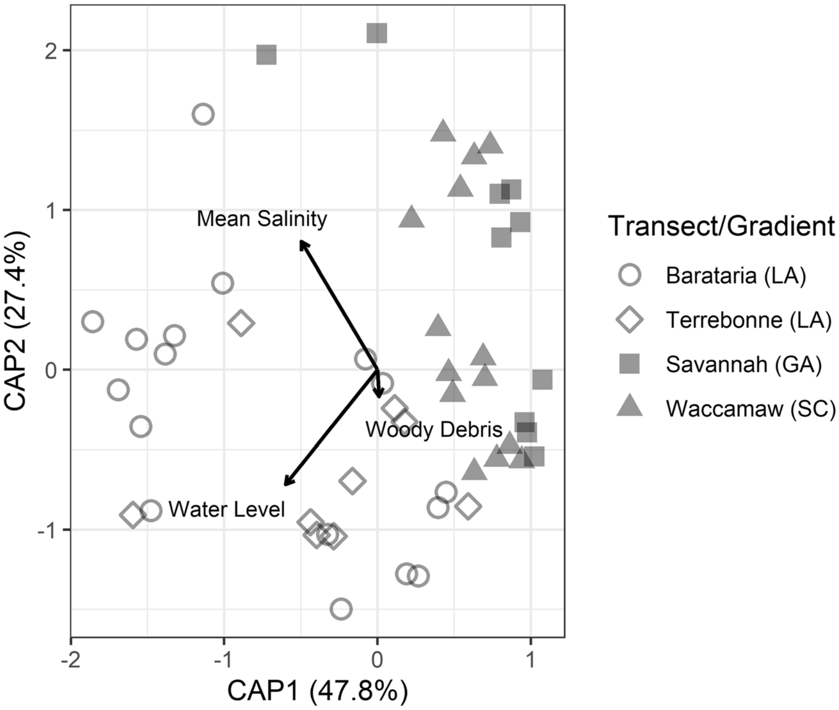
| Endosphere | Rhizosphere |
|---|---|
| Mean Water Level (F = 3.40, $p < 0.001$) | Mean Water Level (F = 5.10, $p < 0.001$) |
| Mean Salinity (F = 3.50, $p < 0.001$) | Mean Salinity (F = 5.10, $p < 0.001$) |
| Woody Cover (F = 1.80, $p < 0.001$ | Woody Cover (F = 2.70, $p < 0.003$) |
| Tree Density (F = 3.02, $p < 0.021$) | |

## Environmental Variables and Community Composition across Taxonomic Levels

Shifts in rhizosphere community compositions in response to salinity occurred across the three taxonomic levels (i.e., class, order and family), while water level appeared to have had less of an influence on changes in composition regardless of the taxonomic level (Table S1). We also observed changes in composition at the order ($R^2 = 0.04$, $p = 0.34$) and family ($R^2 = 0.04$, $p = 0.34$) levels due to space/transect and woody debris volume (Table S1). These shifts were evident in the patterns of the most abundant families (top five) which varied across transects, and across the salinity gradient within each transect. For instance, Bacillaceae was abundant in the rhizosphere across all sites, although at high salinity (<2 ppt), it was less abundant. On the other hand, Gallionellaceae was abundant at only one site (WM in Waccamaw transect in South Carolina, Fig. S3).

Similar patterns were recovered among endosphere communities, with salinity significantly correlating to shifts in

**Fig. 3** In rhizosphere bacterial communities, mean water level, mean salinity, and woody debris volume (woody cover) were selected as significant explanatory variables which explained a significant amount of variation in the model (77.4%) using the Bray-Curtis dissimilarity matrix in a DB-RDA analysis. CAP1 and CAP2 refer to the first and second axes of a Constrained Analysis of Principle Coordinates, output from a distance based redundancy analysis

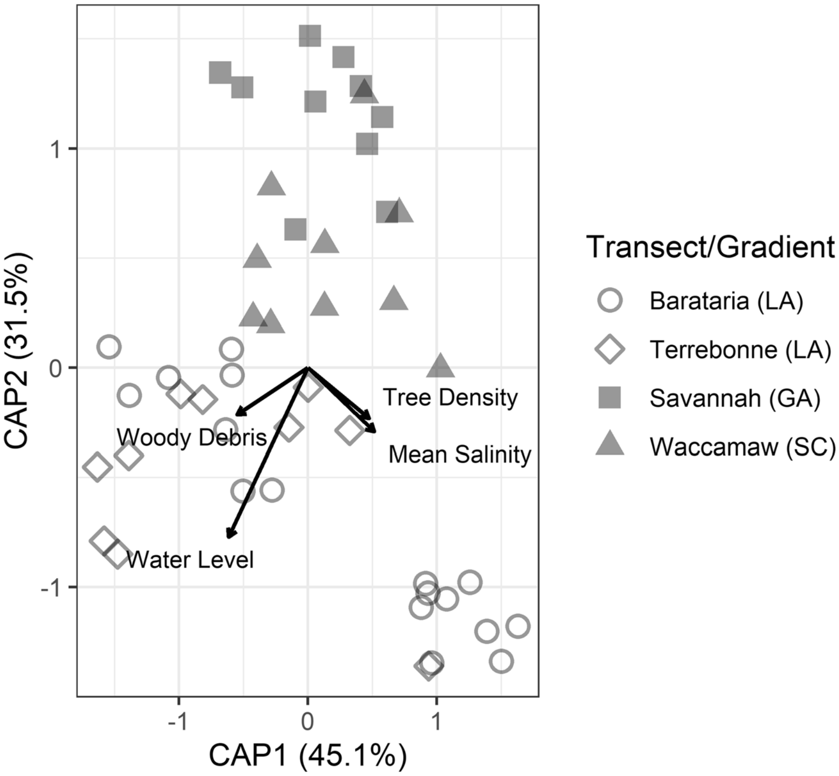


bacterial composition across the three taxonomic levels (Table S2). Shifts in composition at lower taxonomic levels (order and family) were observed due to woody debris volume and along transect (Table S2), which was mirrored by shifts in the abundances of bacterial taxa at the family level (Fig. S4).

## Diversity/Richness/Abundance

Our samples from the rhizosphere ($n = 50$) and endosphere ($n = 47$), contained 5,223,258 sequences, which clustered into 14,377 OTUs of bacteria and archaea. Endosphere

**Fig. 4** In root endosphere bacterial communities, mean water level, mean salinity, woody debris (woody debris volume), and tree density were variables which explained a significant amount of variation (75.4%) in the community composition model using the Bray-Curtis matrix



communities were not a complete subset of the rhizosphere soil bacterial communities, though 40.7% of the overall OTUs were shared between roots and soil. Rhizosphere bacterial communities (mean Shannon diversity = 4.68) were significantly more diverse than endosphere communities (mean Shannon diversity = 4.19; Mann-Whitney U = 654, $p < 0.001$). There were no differences in diversity or richness of microbial communities among sample sites in the endosphere (Kruskal-Wallis H = 7.07, $p < 0.79$) or rhizosphere (Kruskal-Wallis H = 3.64, $p < 0.97$).

The multivariate statistical framework MaAsLin detected significant differences in the relative abundance of several classes of microbial communities in the roots and rhizosphere including, most notably, higher abundances of Alphaproteobacteria (q = 1.72e-37), and Betaproteobacteria (q = 9.64e-11) in the roots compared to the rhizosphere. Additionally, water level was positively correlated with the abundance of Methanobacteria (q = 0.017) and mean salinity was positively correlated with the abundance of bacterial class TK17 (q = 0.019). (A q-value is a $p$ value that has been adjusted for false discovery rates and significant values are less than or equal to 0.05).

## Discussion

Our study examines the relationship of geographic distance and environmental filtering to the community composition of baldcypress root-associated endosphere and rhizosphere bacteria along ecological gradients. We highlight three key findings: 1) community composition is more similar in sites that are closer together in the rhizosphere soil but not in the endosphere bacterial communities; 2) rhizosphere community composition was correlated with mean salinity, mean water level, and woody debris; 3) root endosphere community composition was explained by the same three variables as the rhizosphere and additionally by tree density. However, at the transect level, the influence of these environmental variables on bacterial community composition was unique in different transects.

### Distance Correlated with Rhizosphere but Not Endosphere Bacterial Communities

We found that rhizosphere but not root endosphere bacterial communities were correlated with distance when we used multiple linear regression analyses (MRM) analysis on bacterial community distance matrices regressed against geographic distance. The rhizosphere bacterial communities were more dissimilar the further away they were in physical space. This phenomenon, known as distance decay, has been observed in many studies on soil bacteria (Goldmann et al. 2016; Peay et al. 2010; Martiny et al. 2011). The root endophyte communities showed a similar trend toward distance decay, but the effect of distance was only marginally significant.

The roots of many plants select for/attract specific microbial communities from the rhizosphere by using root exudates, which contain nutrients for the microbes including carbohydrates, organic acids, and amino acids (Compant et al. 2010; Zarraonaindia et al. 2015). It is possible that this recruitment mechanism is occurring for baldcypress and that may have had a stabilizing effect on root endophyte communities, thus decreasing the influence of distance that was observed in the surrounding rhizosphere bacterial communities. Baldcypress trees are genetically diverse (Krauss et al. 1999), so these results might also be an indication that the root interior is a highly selective environment driven by highly conserved genes and pathways or may indicate a stable symbiosis of some microbial taxa with baldcypress trees. An alternative explanation could be some unmeasured variables influencing community structure that masks influence of distance.

### Salinity Correlated with Endosphere and Rhizosphere Communities

We detected a correlation between the mean salinity and the bacterial community composition in both the endosphere and the rhizosphere. It has been well established that increased salinity alters microbial community composition and reduces microbial biomass and activity in the soil (Andronov et al. 2012; Setia et al. 2012; Yan et al. 2015; Thompson et al. 2017). Soluble salts in the soil increase osmotic potential, drawing water out from the cells of microbes and impeding some microbes' ability to acquire water from their surroundings (Oren 1999). Because plants recruit microbes from the rhizosphere and soil that is in closest proximity to the roots, changes in the rhizosphere microbial community due to salinity shifts could be responsible for the observed changes in endosphere microbial communities. It is also possible that salinity indirectly affects microbial communities via tree density and/or woody debris volume, which decreases with increasing salinity (Krauss et al. 2009; this study). Tree density and woody debris can impact organic matter inputs, such as carbon (Krauss et al. 2018), which serves as resource for bacterial communities.

### Water Level Correlated with Endosphere and Rhizosphere Communities

We found that baldcypress endosphere and rhizosphere bacterial communities correlated with mean water level. Bacterial communities could be responding to different environments or to changes in the host plant brought about by environmental change. Though baldcypress can withstand periodic and permanent flooding, prolonged high-water levels can cause stress, and flooding and salt in combination have a known negative interactive effect on baldcypress (Allen et al. 1996). In other systems, stress due to flooding and changes in water regime have been demonstrated to alter the composition and amounts of plant root exudates (Henry et al. 2007; Wang et al. 2017). Stress-induced changes in the plant may influence the root microbiome of the plant by altering its capacity to select for a specific suite of microbes and stress can also increase plants' vulnerability to invasion by pathogens (De Coninck et al. 2015). This was also reflected in shifts in endosphere community composition at higher-level taxonomic groups (i.e., Class and Order, discussed below).

Furthermore, it is known that baldcypress roots adapt morphologically to flooding via the production of water roots and adventitious roots, increasing intercellular air spaces in the roots, and developing shallower root systems (Megonigal and Day 1992). Though we did not systematically record root architecture or appearance for our root samples, we observed that roots collected from flooded/permanently inundated sites were often succulent in texture and/or arose from the trunk closer to the water's surface, as is more characteristic of the "water roots" and adventitious roots described for mature flooded baldcypress (Harms et al. 1980; Megonigal and Day 1992). Rhizosphere soil methods were standardized for each sample; however, it is possible that morphological differences in the roots may have influenced the root microbiome by altering the interior landscape inhabited by microbes.

It is also possible that the flooding conditions selected for a suite of anaerobic microbes which could survive underwater. Our MaAsLin analyses found that the abundance of one class in particular, the Methanobacteria, which are most commonly anaerobic (Chen et al. 2008), was positively correlated with water level. Further explorations are needed into the life histories of specific taxa.

## Woody Debris Volume Correlated with Endosphere and Rhizosphere Communities

Woody debris volume, the volume of downed wood in the layer from soil surface up to 1 m above the soil surface, correlated with shifts in bacterial community composition in the endosphere and the rhizosphere. We note that woody debris volume decreases with increasing salinity, as expected. Bacteria produce extracellular enzymes that degrade lignin/organic matter, and therefore thrive where there is plenty of substrate to decompose (de Gonzalo et al. 2016). Decaying wood provides a substrate for increased bacterial diversity and abundance and thus serves as a local source of microbial dispersal (Jonsson et al. 2012). It follows that an increase in the amount of woody debris volume on a site would serve as fertile fodder for flourishing microbial communities which, in turn, could find their way into the rhizosphere and endosphere. At high salinity where there is less woody debris volume (Krauss et al. 2009; this study) and possibly more baldcypress mortality compared to the freshwater sites, the growth of only a specific suite of bacterial taxa that are capable of utilizing decreased or limited resources were favored.

## Tree Density Correlated with Endosphere Communities

The density of baldcypress trees at each site contributed to the bacterial community composition in the roots but not in the rhizosphere. Distance decay, the decline in community similarity with increasing distance, has been demonstrated in bacterial communities over distances as short as a few centimeters apart (Martiny et al. 2011). Peay et al. (2010) used the framework of island biogeography to examine dispersal limitation of mycorrhizal fungi between individual trees referred to as "tree islands" and found that with increasing isolation of trees, species richness decreased significantly. Given the microscopic nature of endophytes and considering that each baldcypress tree would be a vector for dispersal of baldcypress endophytes, perhaps we are seeing a similar "tree island" effect in our study system, where a greater number of trees in close proximity to one another are influencing community dynamics.

## Shifts across Taxonomic Levels of Rhizosphere and Endosphere Bacteria

Shifts in both endosphere and rhizosphere communities across all three taxonomic levels occurred in response to salinity. This result further affirms that salinity is a major driver of bacterial community composition in wetland ecosystems, promoting growth of specific bacterial groups (León-Lorenzana et al. 2018; Rath et al. 2019). The result shows that there is ecological coherence of salinity preferences by bacterial taxa belonging to phylogenetically more closely-related clades (i.e., Family level) (del Giorgio and Bouvier 2002; Morrissey and Franklin 2015). In contrast, water level was correlated only with shifts in endosphere communities at the higher-level taxonomic groups, indicating that it selects for or favors growth of bacterial taxa across distantly-related clades.

The strong correlation between the biotic factors - tree density and woody debris volume - and shifts in endosphere taxa across higher-level taxonomic groups (Class and Order) highlights the greater influence of host plant attributes on endosphere compared to rhizosphere communities. These results were mirrored by the uniqueness in the top five most abundant families between rhizosphere and endosphere communities. For example, Rhodocyclaceae, which consists of highly disparate members with different modes of living such as anoxygenic photoheterotrophs and plant-associated nitrogen-fixing aerobes (Oren 2014), was highly abundant among endosphere but not in rhizosphere. Meanwhile, Bacillaceae was dominant in rhizosphere but less so in the endosphere.

Thus, specific bacterial groups are favored differently in separate environments (i.e., roots and rhizosphere soil), which is indicative of their unique functions relative to the hosts.

## Limitations/Future Directions

While we did collect geographic coordinates at each 20 × 25 m plot, we did not take coordinate measurements at the individual tree level, which would have allowed for a finer resolution examination of the potential effects of distance decay and dispersal limitation. Additionally, samples were only taken at one time point in each location and were taken several months apart for samples in Louisiana versus the east coast (South Carolina and Georgia). It has been demonstrated that microbial communities shift over time, so both concurrent sampling and longitudinal studies are needed to determine how communities are changing.

## Conclusions

Other studies have noted that salinity and flooding are stressful to baldcypress (Allen et al. 1996). Our work, which is the first to use 16S Illumina sequencing to characterize endosphere and rhizosphere bacteria associated with baldcypress, suggests that stress associated with salinity and flooding (both related to sea level rise) leads to altered microbial communities and that geographic distance has a greater influence in structuring baldcypress associated bacteria in the rhizosphere than in the endosphere. Understanding the ways in which bacterial communities are structured along salinity and flooding gradients in this key restoration species could inform restoration and conservation planning and help us to predict how bacterial communities may shift in dynamic coastal ecosystems.

**Acknowledgements** Samples from the site(s) at Jean Lafitte National Historical Park and Preserve were collected under the National Park Service's permit JELA-2014-SCI-0012. We thank Michael Osland for comments on the manuscript. For help in the field, lab, and with analyses we would like to thank Kimberly Mighell, Peter Tellez, Demetra Kandalepas, Hector Urbina, Mae Berlow, Casey Gu, Kathalina Tran, Max Berdik, Caroline Faircloth, Elaine Broussard, Alison Harrington, Kaylee Arnold, Jennifer Janowsky, Alex Levy, Lorena Torres-Martinez, Rebecca Wang, Tarik Anwar, and Anna Peterson. Funding for S.A.V. and E.K. was from Tulane University's School of Science and Engineering, Louisiana Board of Regents, and National Science Foundation's Graduate Research Fellowship Program. K.K. and R. D. were supported by the U.S. Geological Survey, Land Change Science Climate Research and Development Program. W.H.C. was supported by NIFA/USDA under grant # SCZ-1700531. Technical Contribution No. 6877 of the Clemson University Experiment Station. Any use of trade, firm, or product names is for descriptive purposes only and does not imply endorsement by the U.S. Government.

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